ARGININE EFFECTS ON *IN VIVO* GLUCOSE-INDUCED INSULIN SECRETION IN MICE AND HAMSTERS

Ioana TRANDABURU[1]*, Dorina MIRANCEA[1], Mirela SIMIONESCU[1], Tiberiu TRANDABURU[2]
[1]Centre of Cytobiology, Institute of Biology, Bucharest
[2]Laboratory of Histology and Embriology, Faculty of Sciences, University of Pitesti

*** Correspondence:** Ioana TRANDABURU, Institute of Biology, no. 296 Spl. Independenţei, 060031, Bucharest, e-mail: itrandaburu@yahoo.com; ioana.trandaburu@ibiol.ro
Received: march 2008; Published: may 2008

ABSTRACT. Alterations of the biphasic pattern of insulin secretion and of glycemia induced with glucose were described for the first time in hamsters after the sole i.p. injection of a mixture of glucose (3,3 g glucose/kg body weight) and L-arginine (7,16g arginine/kg body weight) or after 10 min. delayed injections of identical amounts of the same hexose and amino acid . With the exception of several minor, species-specific differences, the monophasic hyperglycemic profiles evoked by both experimental variants pledge undoubtedly for a stimulatory effect of arginine on the glucose induced insulin secretion (time-dependent potentiation; TDP), much less expressed in mice than in hamsters. Taking into account, however, the quantitative fluctuations of the B-cells granular contents evinced histochemically is rather likely to presume the co-existence and successive exercise of both potentiation and inhibition states (TDP, TDI) of the glucose-induced insulin secretion in the two rodents species studied. The above results obtained "in vivo" are discussed in connection with the findings reported in other mammalian species.

Keywords: *in vivo* time-course, insulin secretion and glycemia, arginine and glucose, mice and hamsters

INTRODUCTION

The mechanism through L-arginine affects "in vitro" the biphasic dynamic of the glucose-induced insulin secretion was elucidated by Thams and Capito still from 1990. Before them, but also more recently, an investigations series "in vitro" a have been addressed to the comparative effects of the same and other amino acids (leucine, glutamine) on the glucose-stimulated hormone secretion both in mice and hamsters (Nesher et al., 1984 a,b; Zawalich et al., 2004). Considering the above, Nescher and Cerasi (2002) have been the first who advanced the opinion according to which the arginine, as a non-nutritive secretagogue of the B-cell, could evoke an inertness state of insulin secretion irrespective of the duration of hexose stimulation. This state is known at the present day as time-dependent inhibition (TDI).

In this paper, the biphasic dynamics of glycemia and of insulin secretion induced "in vivo" by the glucose administration in mice, such as those recently demonstrated by us (Trandaburu et al., 2006) and other authors (Henquin et al., 2002; Nunemaker et al., 2006), occur drastically altered through the loss of the second hyperinsulinic, respectively hyperglycemic phase. The demonstration of such modifications provoked by the injection of L-arginine, involving the alterations of insulin release from the pancreatic B-cells of hamsters, has represented undoubtedly the main aim of the present paper. In other words, we have proposed ourselves to render evident for the first time "in vivo" the stimulatory (TDP) or inhibitory (TDI) actions of L-arginine on the glucose-induced insulin secretion in hamsters. Subsidiary, we have proposed ourselves to compare "in vivo" the dynamics of glycemia and of the secretor response of B-cells in both rodent species (mice and hamsters) under study.

MATERIAL AND METODS

A total number of 30 *Mus musculus* mice and 24 *Mesocricetus auratus* golden hamsters aging three, respectively four month, have been used. Each experimental group, as well as the control groups comprised 5 mice and 4 hamsters.

The experimental pattern I: the animals were injected i.p. with a sole dose of glucose and L-arginine (3.3g glucose/kg body weight + 7.16g L-arginine/kg body weight) and were sacrificed by decapitation 10, 30, 45, 90 and 120 min. after the mixture administration.

The experimental pattern II: The animals were injected i.p. with identical doses of glucose (3.3g glucose/kg body weight) and L-arginine (7.16g/kg body weight) 10 min. time-delayed. The mice and hamsters were killed by decapitation at the same time-periods (10, 30, 45, 90 and 120 min) after the glucose administration.

At the pre-established time-periods the plasma glucose levels of all individuals (mice and hamsters) were counted with an Optium Xceed glucometer (Abbott Labs., Abingdon, UK) and the pancreases were removed for histochemical investigations. The glycemic values of each treated and control rodent groups were expressed as means ± standard deviations (M ± SD).

The pancreatic organ of the mice and hamsters was fixed in Bouin's fluid for at least 36 h and was further

processed for paraffinwax-embedding using routine protocols. 6 µm-thick sections, obtained with a sledge microtome (E.Leitz, Wetzlar, Germany), were mounted on poly-l-lysine (Sigma, St.Louis, Mo, USA) or gelatine-coated glass slides. They were stained metachromatically with pseudoisocyanine (N,N-diäthylpseudoisocyanin HCl, Serva, Heidelberg,Germany) according to the technique initiated by Coalson (1966). The preparates were examined and photographied in monochromatic light of 580 nm (Epple, 1966).

RESULTS AND DISCUSSIONS

According to the opinion of Nescher and Cerasi (2002), the inertness state of the insulin secretion (TDI) following the supplementation of the culture medium with arginine persists a considerable time-period after the amino acid removal. By this effect, the arginine planishes the profile of the second phase of hormone realizing in blood ascribed to its biosynthesis. In this case the biphasic dynamics of the glucose-induced glycemia, recently demonstrated "in vivo" by several authors (Henquin et al., 2006; Nunemaker et al., 2006) in mice and also by us (Trandaburu et al., 2006, -in print) in hamsters, appear completely modified by the disappearing of the second hyperglycemic peak. The effect was possible by unique administration of a mixture of glucose and L-arginine (experimental pattern I) illustrated in Chart Ia, in which it is also inserted the biphasic dynamic of the glucose-induced glycemia.

Recently, Ishiyama et al (2006) established that the glucose stimulates "in vitro" the insulin secretion induced with arginine or with tolbutamide, most probably through a dual mechanism involving both increasing of the Ca^{2+} ions concentration in the cytosol of B-cells and their stimulatory action on the hormone exocytose. According to the same authors, the effect of hexose potentiation on insulin secretion (TDP) would involve alterations of its action mechanism at Ca^{2+} ions level. The above findings, as well as the beneficial effects of the oral L-arginine treatment added to a hypo caloric diet and exercise training program of the obese type - 2 diabetic patients (Lucotti et al., 2006), determined us to perform the experimental pattern II on mice, in which was detected the disappearance of both hyperglycemic peaks following 10 min delayed injections of L-arginine and glucose (Chart Ib). In the above conditions, the slight and unique glycemic elevation appeared more persistent and normalized itself at about 80 min. after the amino acid, respectively 90 min. after the glucose injection.

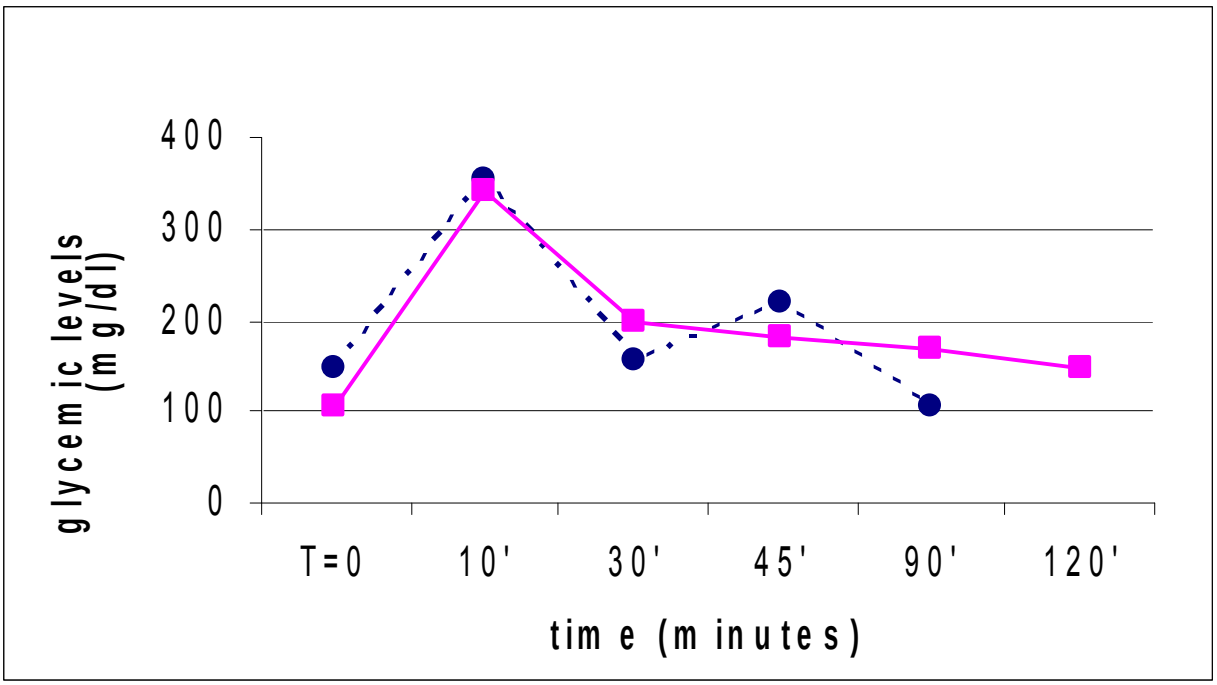


Chart Ia Comparison of the glycemic dynamics in mice induced by the sole i.p. injection of glucose (dotted line) and of a mixture of glucose and L-arginine (continuous line)

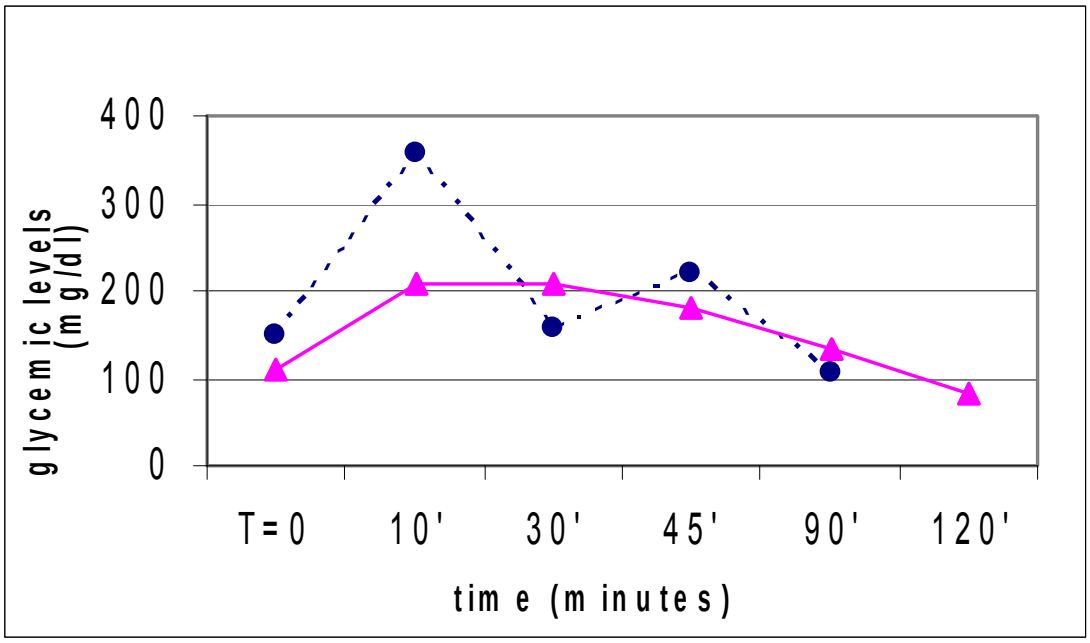


Chart Ib Comparison of the glycemia dynamics in mice following a singular i.p injection with glucose (dotted line) and two 10 min. delayed injections, the second one with L-arginine (continuous line)

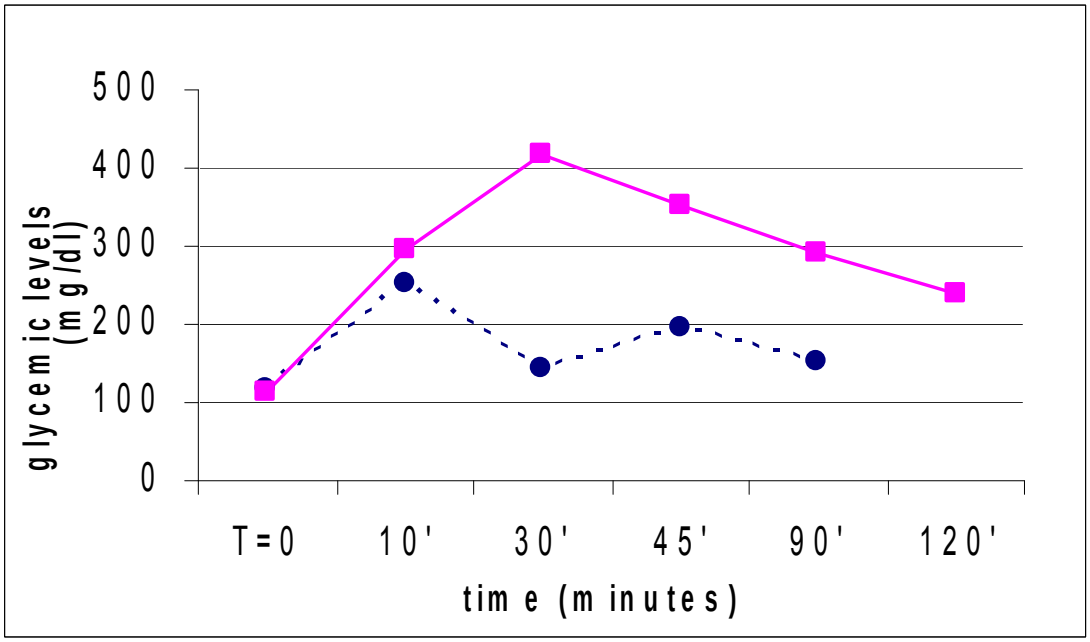


Chart IIa Comparison of the glycemic dynamics in hamsters induced by the sole i.p. injection of glucose (dotted line) and of a mixture of glucose and L-arginine (continuous line)

The singular administration "in vivo" of glucose and L-arginine mixture induced in hamsters a single hyperglycemic profile, but with the double amplitude comparative with the both hyperglycemic phases generated by the unique administration of the same glucose quantity (Chart IIa).

The monophasic hyperglycemic profile obtained in these rodents distinguishes from the one recorded in mice (Chart Ia), both by the delay in time of the maximum values (10 min. in mice; 30 min. in hamsters), as well as by the persistence of the hyperglycemic answer at 120 min. from the combined injection of glucose plus amino acid (answer which is almost normoglycemic in mice and double normoglycemic in hamsters). The monophasic profiles induced by the unique administration of a glucose and L-arginine mixture in mice and hamsters (Chart Ia and IIa) also differ from each other by their time extension 30 min. in mice and over 120 min. in hamsters. If we have in mind also the fact that the unique hyperglycemic peak in hamsters overleaps (30 min.) the nadir between the two hyperglycemic phases induced by the singular glucose administrations (Chart IIa), it is highly possible that we are dealing with an

arginine potentiation of the glucose-induced insulin secretion (TDP).Such an arginine effect appears pregnant also in the case of separate amino acid administration, delayed with 10 min. from that of glucose (Chart IIb), situation in which is observed at 30 min. more than double of normoglycemic values. According to Nesher and Cerasi (2002), the arginine effect would correspond to the co-existence and exercise with different weights on the B-cells of the inhibition (TDI) and potentation (TDP) states of the glucose-induced insulin release.

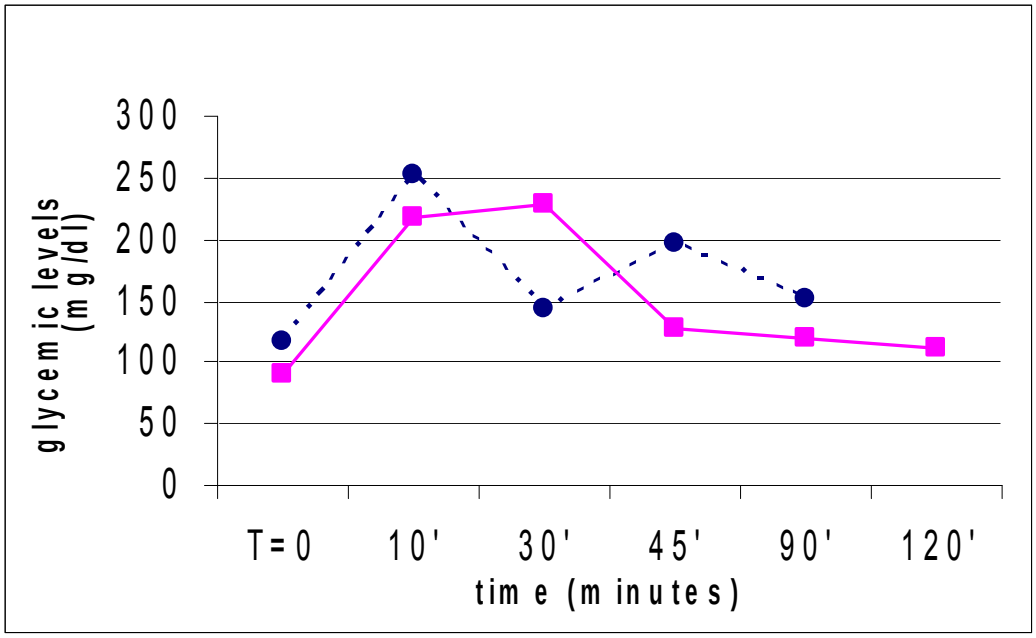
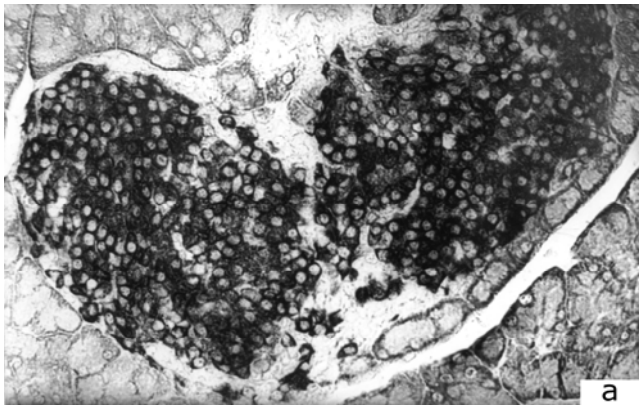
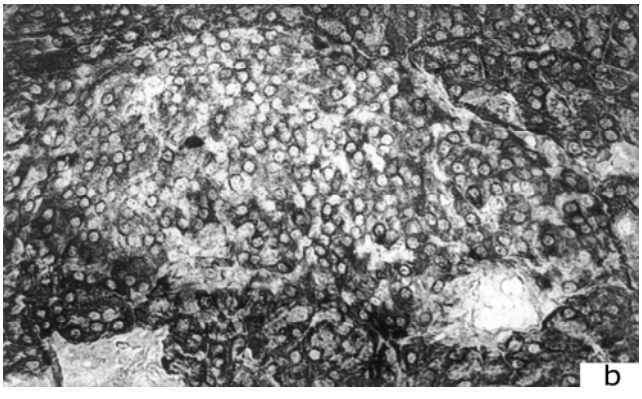


Chart IIb Comparison of the glycemia dynamics in hamsters after one i.p.injection with glucose (dotted line) and two, 10 min. delayed injections, the second one with L-arginine (continuous line)

The histochemical relievance with pseudoisocyanine of the B-cells granular content, roughly confirms the monophasic dynamics of the glycemia recorded in mice (Chart Ia and Ib) and hamsters (Chart IIa and IIb), under the conditions of the singular administrations of glucose and L-arginine, combined (Chart Ia and IIa) or in the ones in which the L-arginine injection was followed after 10 min. from that of glucose (Chart Ib, IIb). The quantitative variations of the secretory granules amounts from these cells seem to proceed, at least with several minutes, the development of monophasic patterns of glycemia both in mice (Fig. 1 a-f) and hamsters (Fig. 2 a-f).





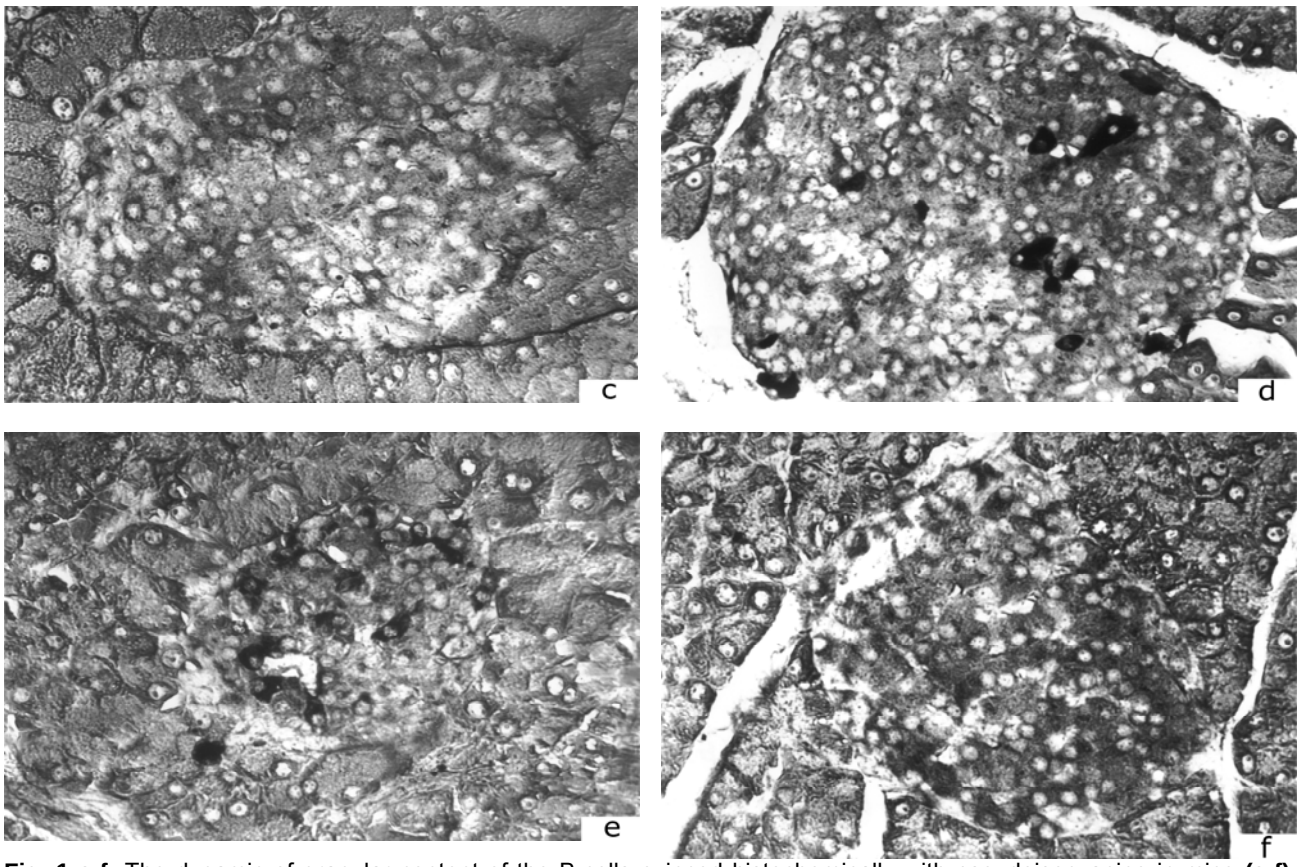
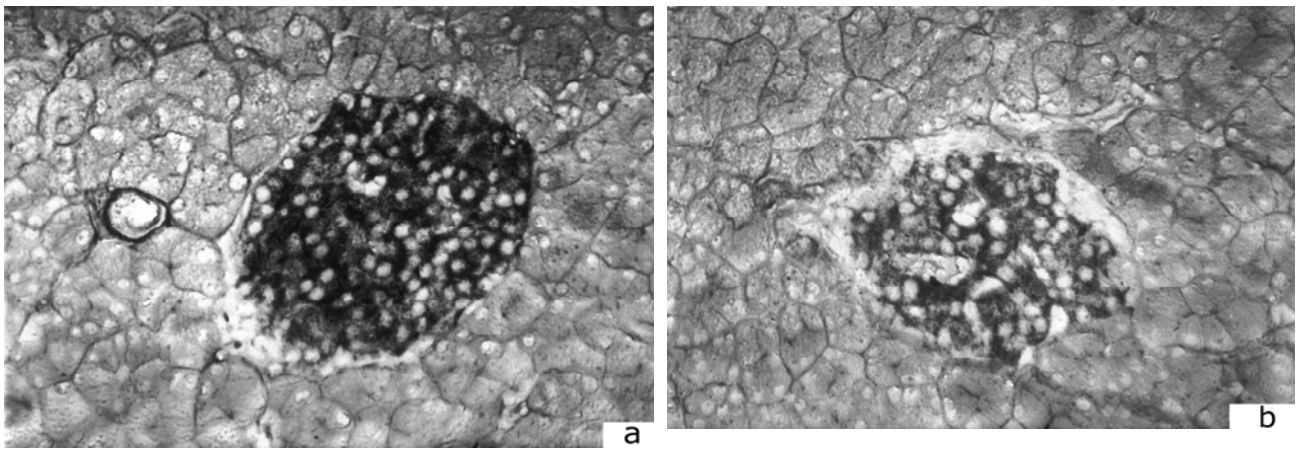


Fig. 1 a-f: The dynamic of granular content of the B-cells evinced histochemically with pseudoisocyanine in mice **(a-f)** injected with an unique dose of glucose and arginine (experimental pattern I). To note the most advanced degranulation degree of this cells type at 10 min **(b)** after the mixture administration and the reloading trend of these cells **(c-f)**

In addition, should be mentioned that the most pronounced degranulations of the insulin producing cells were noticed at 10 min. after the glucose injection in mice (Fig. 1b) and at 30 min. (Fig. 2c) in hamsters, both time-intervals corresponding to the hyperglycemic peaks induced by the combined administration of the glucose and amino-acid. At the next time-intervals (45 min, 90 min, 120 min), the granular contents of the B-cells followed the some increasing trends, quite slower in mice and far more pronounced in hamsters (Fig.1d-f; Fig.2d-f).



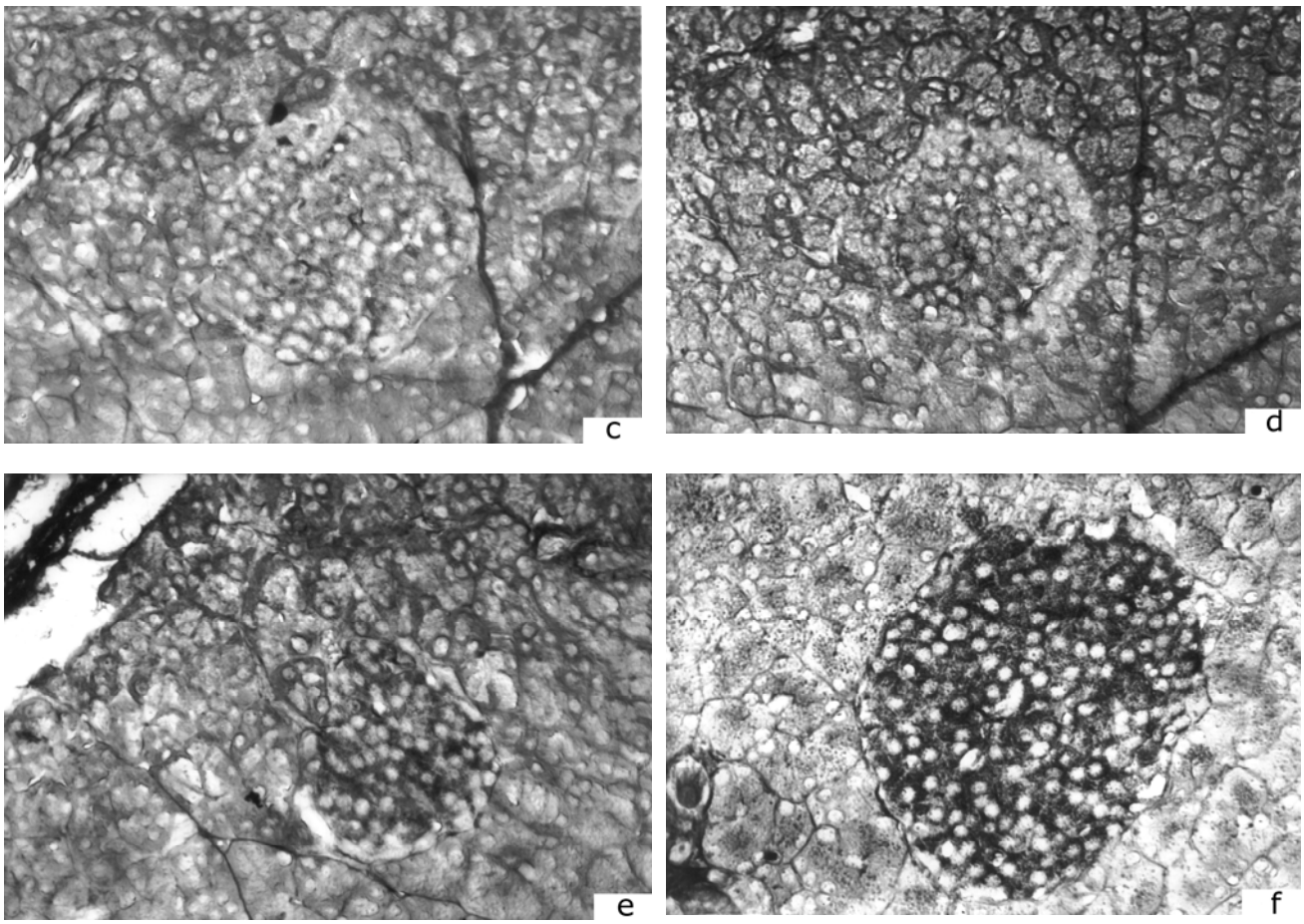


Fig. 2 a-f: The dynamic of granular content of the B-cells evinced histochemically with pseudoisocyanine in hamsters **(a-f)** injected with an unique dose of glucose and arginine (experimental pattern I). To observe in this rodent species the movement of the most pronounced degranulation degree of B-cells at 30 min **(c)** after the mixture injection, as well as their slowly reloading trend **(d-f)**

Special attention should be paid to the lack of synchronization of hormone release among B cells of an islet and also among the islets of an individual. Reported previously "in vitro" in humans and several rodent species (Rorsman et al., 2000; Straub and Sharp, 2004) and also "in vivo" (Trandaburu et al., 2006), the cellular and insular desynchronizations of insulin release could explain, at least partly, the individual variations of glycemia induced by a single, combined or time-delayed dose of glucose and arginine .

Finally, the differences concerning the histochemical expression of the B-cells and the glycemia levels 120 min. after glucose and arginine injections or only 90 min. after the sole glucose administrations (Trandaburu et al., 2006), all might be ascribed to a genetic species-specific substrate (Zawalich and Zawalich, 1996; Zawalich et al., 2001).

CONCLUSIONS

L-arginine injected simultaneously with glucose evokes a time-dependent inhibition TDI) of insulin secretion induced with this hexose in mice and hamsters.

The same amino acid injected 10 min. after the glucose administration elicits a time-dependent potentiation (TDP) of insulin secretion in both rodent species under study.

In spite of the interspecific peculiarities of hormonal responses (reaction speed) and of glycemia dynamics, L-arginine seems to exercise "in vivo" a modulator effect on insulin secretion-induced by glucose.

The present paper emphasized for the first time that in hamsters the biphasic pattern of insulin secretion-induced with glucose might be modulated "in vivo" by arginine administration.

REFERENCES

Zawalich W.S., Zawalich K.C. Species differences in the induction of time-dependent potentiation of insulin secretion. Endocrinology 1996;137: 1664-9.

Thams P, Capito K. L-arginine stimulation of glucose-induced insulin secretion through membrane depolarization and independent of nitric oxide. Eur.J.Endocrinol.,1999;140: 87-93.

Zawalich W.S., Yamazaky H., Zawalich K.C., Cline G. Comparative effects of amino acids and glucose on insulin secretion from isolated rat or mouse islets. J.Endocrinol., 2004;183: 309-19.

Nesher R., Cerasi E. Modeling phasic insulin release: immediate and time-dependent effects of glucose. Diabetes 2002;51, supl 1: 53-9.

Nesher R., Tuch B., Hage C., Levy J., Cerasi E. Time-dependent inhibition of insulin release: suppression of the arginine effect by hyperglycaemia. Diabetologia 1984a;26: 142-5.

Nesher R., Waldman L., Cerasi E. Time-dependent inhibition of insulin release: glucose-arginine interactions in the perfused rat pancreas. Diabetologia 1984b;26: 146-9.

Henquin J.C., Ishiyama N., Nenquin M., Ravier M.A., Jonas J.C. Signals and pools underlying biphasic insulin secretion. Diabetes 2002;51, suppl 1: 60-7.

Nunemaker C.S., Wasserman D.H., McGuinness O.P., Sweet I.R., Teague J.C., Satin L.S. Insulin secretion in the conscious mouse is biphasic and pulsatile. Am.J.Physiol.Endocrinol.Metab. 2006;290: E523-9.

Coalson R.E. Pseudoisocyanine-staining of insulin and specificity of empirical islets cell stains. Stain Technol. 1966;41: 121-131.

Epple A. Islet cytology in urodele amphibians. Gen.Comp.Endocrinol. 1966;7: 207-14.

Trandaburu I., Mirancea D., Argint M., Trandaburu T. Biphasic dynamic of the "in vivo" glucose-induced insulin secretion in mice and hamsters. Rom. J.Biol.- Zool. 2006; 51 in print

Lucotti P., Setola E., Monti L.D., Gallucio E., Costa S., Sandoli E.P., Rabaiotti G., Gatti R., Piatti P. Beneficial effects of oral L-arginine treatment added to a hypocaloric diet and exercise training program in obese, insulin resistant type 2 diabetic patients. Am.J.Physiol.Endocrinol.Metab. 2006; 29:E906-12.

Ishiyama N., Ravier M.A., Henquin J.C. Dual mechanism of the potentiation by glucose of insulin secretion induced by arginine and tolbutamide in mouse islets. Am.J.Physiol.Endocrinol.Metab. 2006; 290:E540-9.

Zawalich W.S. Zawalich K.C., Tesz G.I., Sterpka J.A., Philbrick W.M. Insulin secretion and IP levels in two distant lineages of the genus Mus: comparisons wit rat islets. Am.J.Physiol.Endocrinol.Metab.,280:E720-9, 2001

Rorsman P., Eliasson L., Renstrőm E., Gromada J., Barg S., Gőpel S.The cell physiology of biphasic insulin secretion.News Physiol. Sci. 15, pp. 72-77, 2005

Straub S.G., Sharp G.W.G. Hypothesis: one rate limiting step controls the magnitude of both phases of glucose-stimulated insulin secretion. Am.J.Physiol.Endocrinol.Metab. 287, pp. 565-71, 2004

Studia Universitatis "Vasile Goldiş", Seria Ştiinţele Vieţii (Life Sciences Series), vol. 18, 2008

http://www.studiauniversitatis.ro